COMPARISON OF THE EFFECTIVENESS OF STERILIZING ENDODONTIC FILES BY FOUR DIFFERENT METHODS – AN INVITRO STUDY

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CERTIFICATE

This is to certify that this dissertation titled **"COMPARISON OF THE EFFECTIVENESS OF STERILIZING ENDODONTIC FILES BY FOUR DIFFERENT METHODS – AN INVITRO STUDY"** is a bonafide record of work done by **Dr. R. Venkatasubramanian** under my guidance during his post graduate study period between 2001-2004.

This Dissertation is submitted to THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, in Partial fulfillment for the Degree of **Master of Dental Surgery in Branch VIII - Pedodontics and Preventive Dentistry.**

It has not been submitted (partial or full) for the award of any other degree or diploma.

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CONTENTS

INTRODUCTION

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Microorganisms induce a variety of infections and diseases in the human body and are largely ubiquitous in nature. Contamination directly or indirectly leads to transmission of infectious agents.[6]

The recent increase in the knowledge and the information on the transmission of the virus of the B hepatitis (HBV) and the virus of the immunodeficiency human being (HIV) has given new emphasis to the problem of the contamination crossed during the dental treatment.

Infection control is a major issue in medicine and dentistry because of concern over communicable diseases transmitted in health care settings. The prevention of cross-contamination of infectious diseases among dental staff and patients is a major concern in a dental practice.

In 1987, the Center of Disease Control (CDC) called the term “Universal Precaution” as being the set of procedures and measures that they aim at to protect the health and to provide security to the professionals of the area and, therefore, to the patients. These Universal Precautions understand the job of barriers of surfaces, sterilization of the instrument of clinical use, antisepsis, disinfection, cleanness and discarding of dismissable materials.

Sterilization is the best method to counter the threats of microorganisms. The purpose of sterilization in health care field is to prevent the spread of infectious diseases. In dentistry, it primarily relates to processing reusable instruments to prevent cross infection[26].

Sterilization is the procedure that kills all microorganisms, including spores, which are the most difficult types of microbes to eliminate. If the sterilization process has been performed properly, absolutely no microbial life will exist.

International Convention of Endodontia held in Philadelphia in 1958 pointed out the necessity of sterilization of the endodontic instrument for success of the endodontic treatment.

The sterilization of the endodontic rasps is important for two reasons: it controls cross infection and it increases endodontic successes.

In endodontics various instruments like files, reamers, gates glidden drill and peeso reamers are used for cleaning and shaping the root canal system and to eliminate the bacterial population in pulp canal space. Various methods are followed to sterilize these instruments such as dry heat sterilizer, autoclave, ethylene oxide gas, glass bead sterilizer or hot salt sterilizer etc.

The Council on Dental Therapeutics, Council of Dental Materials, instruments and equipment and Council on Dental Practice

recommend the chair side use of glass bead sterilizer for sterilization of intra-canal instruments since it provides rapid sterilization at a temperature of 415°F – 425°F[7].

The Council of Dental Therapeutics also recognizes liquid preparation of formaldehyde as high-level disinfectant and glutaraldehyde as sterilizing agent[7].

Steam under pressure (autoclave)

The conventional autoclave, uses hot steam (250°F or 121°C) in a pressurized chamber to sterilize items and the required exposure time is approximately twenty minutes at fifteen pounds pressure of steam per square inch.

Lasers

CO_2 laser can be used to sterilize endodontic reamers. The living bacteria in tissue can be quickly vaporized and destroyed by CO_2 laser beam. Adrian and Gross[1] have shown that CO_2 laser is capable of effectively sterilizing a scalpel blade at 10 watts in the continuous mode for 1.5 to 2 minutes each by moving the laser beam all over the visible surface in a sweeping motion, after previous contamination with spores. CO_2 laser is reflected by metal surfaces, but the biological tissues absorb virtually all the CO_2 laser beam resulting in their vaporization.

Chemical sterilization

Glutaraldehyde products are the only chemicals that are sufficiently sporicidal to accomplish sterilization. Exposure time is long (6 – 10 hours). During this period of immersion, no other instruments may be added or removed from the bath, as this interrupts the process. Because of toxicity, glutaraldehyde baths must be covered and left in areas with good ventilation. Glutaraldehyde must be thoroughly rinsed off instruments with sterile water before they are used. Glutaraldehyde (greater than or equal to 2%) also kills poliovirus rapidly, but require extensive time periods to kill the tuberculosis bacteria (40 to 60 plus minutes).

Few studies have been done in sterilizing endodontic files by comparing different methods. Hence the study was undertaken with the following aims and objectives.

AIMS AND OBJECTIVES

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The purpose of this study is to compare and recommend the effective method of sterilizing endodontic files in dental practice.

The aim of this study is to investigate the efficacy of four accepted methods of sterilizing endodontic instruments:

(1) Autoclaving

(2) Carbon dioxide laser sterilization

(3) Chemical sterilization – Glutaraldehyde

(4) Glass-bead sterilization

REVIEW OF LITERATURE

REVIEW OF LITREATURE

Hubbard Jr. et al[15] in 1975 did a study to find the effective method of chair side decontamination of endodontic files. The files were contaminated with either Bacillus subtilis or Streptococcus mitis. They were subjected to various decontamination procedures like wiping with a dry gauze sponge, wiping with gauze soaked with isopropyl alcohol or sterile saline or glutaraldehyde solution, rasping the files in a dry cellulose sponge, and by placing it in bead sterilizer for 10 seconds at 425°F. Five files were subjected to each decontamination technique. The study showed that all gauze wipings showed reductions of microorganisms greater then 90 percent. The bead sterilizer showed reduction of 98.9% for B.subtilis spores and 100% for S.mitis. Rasping the file into a dry sponge showed the least amount of reduction of spores, only 35.5% for B.subtilis and 78.5% for S.mitis. The study concluded that glass bead sterilizer was found to be the most effective decontamination method.

Younis[37] in 1977 did a study to see the effect of different sterilization techniques on the properties of intracanal instruments. 504 endodontic reamers and files, both carbon and stainless-steel instruments were used, of which 420 were sterilized by three common sterilization techniques – autoclave (15 and 25 min at 250°F), salt sterilization (5 and 10 seconds at 480°F) and dry heat (1 hour at

340°F). The remaining eighty-four endodontic instruments were tested without being subjected to any sterilization techniques and served as controls. The study showed that carbon steel intracanal instruments were affected more by the different modes of sterilization than were the stainless steel instruments. The time of sterilization did not have an effect on the properties of either instrument.

Adrian C.J & Gross A[1] in 1979 studied the use of the carbon dioxide laser (CO_2) for sterilization of metal instruments. Twenty-six No. 15 scalpel blades were divided into two equal groups of 13 each, of which one group was contaminated with Bacillus subtilis spores and the other group was contaminated with Clostridium sporogenes. Three from each group was not sterilized and taken as control group. Ten from each group were exposed to CO_2 laser system at 10 watts in the continuous mode for 1.5 to 2 minutes each by moving the laser beam all over the visible surface in a sweeping motion. The blades were then placed in tubes containing thioglycollate medium and incubated for 21 days at 37° C. The results showed that none of the tubes containing sterilized blades showed any growth, but all the controls showed growth.

Hooks et al[14] in 1980 studied a new method of sterilizing endodontic instruments by using a carbon dioxide laser system. One hundred reamers were divided into Five groups, each containing 20 reamers. Group A and B were contaminated with Bacillus subtilis var

niger spores. Group C and D were contaminated with Bacillus stearothermophillus spores. Group E was used as the sterile control. Groups A and C were used as positive controls to ensure proper contamination by the spores. Groups B and D were irradiated for 3 seconds per surface at 10 watts using the CO_2 laser system by moving the beam along the length of the instrument during the 3 –second period for per surface exposure. The shafts of the reamers in all the groups were removed from the handle by means of a sterile hemostat and placed into a test tube containing thioglycollate medium. Groups A and B were incubated at 37°C for 21 days, after 21 days, they were heat-shocked at 80°C for 20 minutes and reincubated for 3 days. Groups C and D were incubated at 55°C for 24 days. In Group E, 10 reamers were incubated at 37°C and remaining 10 reamers were incubated at 55°C. The results indicated that there was no growth in sterile group and those irradiated with CO_2 laser beam, whereas all the contaminated controls showed growth. The study concluded that CO_2 laser is an effective way to quickly sterilize endodontic reamers.

Parkes et al[24] in 1982 did a study to see the effect of sterilization on the cutting edges of periodontal curets and scalers. New sharpened carbon and stainless steel instruments were used. The instruments were sterilized by any of the three different sterilization techniques – saturated steam sterilization at 250°F, formalin-alcohol vapor at 270°F, and dry heat up to 340°F. The instruments were

evaluated before and after sterilization with a scanning electron microscope. The study showed that there was no damage to the cutting edges of stainless steel instruments. Carbon steel instruments were not damaged by formalin-alcohol vapor or dry heat sterilization, but caused oxidation and dulling after steam sterilization.

Mitchell B.F et al[18] in 1983 studied the effects of cyclic autoclave sterilization and simulated clinical usage on the torsional behavior of stainless steel endodontic files. The files were divided into six groups, with sixty files in each group. Each group consisted of ten identical files for each of the sixes tested. The different sizes used were size 15, 20, 25, 30, 35 and 40. Three groups served as controls and three were experimental. Extracted human maxillary central incisors, lateral incisors and canines were used in the clinical simulation. The files were sterilized before use. All the groups were used in the biomechanical cleansing and shaping with pure filing motion circumferentially for 10 seconds of the root canals with 5.25% sodium hypochlorite as irrigant. After instrumentation, all files were ultrasonically cleaned for 12 minutes and stored. The experimental groups were autoclaved to heat at 275°F, held for 20 min, and then dried rapidly with forced exhaust. One control and one experimental group were subjected to torsional testing and the handle of each file was removed at the point of attachment. The testing device for all torsional tests is the Torqumeter Memocouple, recording at 2 r.p.m, recording the degree of rotation on a digital display or strip chart

recorder. The same process was repeated for the remaining two control and experimental groups and were cyclically autoclaved five times and then tested for torsion. The same process was repeated for the remaining two control and experimental groups and were cyclically autoclaved five times and then tested for torsion. The results indicated that the repeated autoclave sterilization of stainless steel endodontic files results in a reduction in the number of degrees of angular deflection. This occurred after the fifth exposure to autoclave. It also showed that sizes 35 and 40 were the most adversely affected by the steam-under-pressure sterilization. The study concluded that repeated sterilization of a stainless steel endodontic file results in a significant reduction in the torque resistance of that file, but however that reduction is not significant clinically.

Fahid et al[8] in 1984 studied to determine the effect of cleaning endodontic files with either dry gauze or alcohol-saturated gauze prior to placement of the files into a hot bead sterilizer. The study indicated that an alcohol wipe was more effective than a dry wipe. It also suggested that using an alcohol wipe and 3 seconds in a hot bead sterilizer for No. 10 files or 5 seconds for either a No. 30 or a No. 45 file was equivalent in disinfecting ability to 8 seconds and a dry wipe using the same file sizes.

Rueggeberg et al[28] in 1988 tested the mechanical properties of endodontic broaches before and after hot bead sterilization. Endodontic broaches from three manufacturers were tested for torsion and angular deflection before and after hot bead sterilization. The torsion and angular deflection at torsional failure increased after sterilization. The study concluded that heating affected both torsion and bending.

Morrison et al[20] in 1989 studied to see the effect of steam sterilization and usage on sharpness on size 25 endodontic files. Fifty-five files were divided into eight groups, five groups as controls each containing five files each and the remaining three groups as experimental each containing ten files each. The experimental group files were used to instrument 1, 5, and 10 molars and the control groups determined the effect of repeated steam sterilization on cutting efficiency of unused files. A cutting efficiency test was performed and scanning electron microscopic analysis was performed randomly by choosing one file from each control group and two files from each experimental group. The results showed that there was significant difference in cutting efficiency between experimental files used to instrument 1 molar and those used for 5 or 10 molars. The difference in cutting efficiency between the experimental files used to instrument 5 or 10 molars was not significant, indicating that most of the

decrease in sharpness occurred with use between one and five molars. No significant difference was found between the control groups, indicating no decrease in cutting efficiency by repeated sterilization alone. The study concluded that the primary decrease in cutting efficiency occurs with file usage in one to five molars and the SEM confirmed this.

Murgel C.A.F et al[21] did a study in 1990 to quantitate and compare debris remaining on endodontic files after cleaning. One hundred ten files were used and divided randomly into eight groups. The control group consisted of negative (no usage, no cleaning) and positive (usage, no cleaning). The experimental group consisted of immediate cleaning: gauze/alcohol 15 files, sponge/alcohol 15 files, ultrasonic bath 15 files, and 1-hour delay cleaning: gauze/alcohol 15 files, sponge/alcohol 15 files, ultrasonic bath 15 files. All the groups except negative control group were used for 4 min in a filing action in the canals of extracted teeth along with distilled water irrigation. Following instrumentation all files were subjected to one of the three methods of cleaning: sponge with alcohol, gauze with alcohol and ultrasonic bath for 5 minutes. The cleaning methods were tested immediately and 1-hour after delay. The results showed that the cleaning effectiveness was similar in both gauze/alcohol and ultrasonic bath, but sponge/alcohol was the worst cleaning method. The 1-hour delay before cleaning did not affect the cleaning method

and its effectiveness. The study concluded that none of the methods tested were able to totally clean the files.

Powell et al[25] in 1991 did a study to compare the ability of three lasers (argon, CO_2, and NdYAG) to sterilize dental instruments. Endodontic reamers were contaminated with microorganisms and were exposed to laser system at various levels of energy, placed in Trypticase soy broth, incubated and growth checked to determine sterility. The results indicated that argon laser is capable of sterilizing at the lowest energy level (1 watt for 120 seconds) of the three lasers tested. The other two lasers were also able to sterilize the instruments, but at higher energy levels. The study concluded that all three lasers were capable of sterilizing dental instruments.

Luper et al[17] in 1991 did a study to investigate the effect of different sterilization methods on the fatigue life of finger pluggers. One hundred pluggers were used of which 10 were not sterilized and used as control group. Ninety finger pluggers for each of the four sizes (A, B, C, and D) were subdivided into subgroups of 10. Each subgroup was subjected to 1, 8 or 15 cycles of steam autoclave, dry heat or bead sterilization. Then all the pluggers including the control group was subjected to cyclic bending until fracture. The study showed that only the A finger pluggers autoclaved for eight cycles had a significant lower number of cycles to failure compared with that of controls. Nine subgroups had significant greater number of cycles before failure than

the control. The study concluded that any of the three sterilization methods could be used without fear of plugger failure.

Nammour et al[22] in 1991 did a study to test the sterilizing potential of CO_2 laser. Two turbines and 70 stainless steel strips were prepared. Five microns of suspensions (microbial and salivary) were set and exposed to laser beam at 5 W for 20 seconds. It was tested for sterilization both before and after drying of the suspension. The results showed that sterilization was complete both before and after drying the suspension. The study concluded that CO_2 laser had a important potential for sterilization.

Boyd et al[3] in 1994 did a study to evaluate the sterility of files and spore strips following autoclaving in a sponge. Commercial spore strips and contaminated endodontic files were inserted into sponges, sealed in sterilization pouches and autoclaved. The spore strips and the files were removed from the sponge and cultured for growth of microorganisms. The results showed that no microbes were cultured from spore strips or contaminated files after autoclaving them in the sponges sealed in autoclave pouches. The study concluded that the insertion of files into the sponges used in this study does not obstruct the autoclaving process.

Stach et al[31] in 1995 did a study to investigate the effect of repeated ultrasonic cleaning, cycles of autoclave or chemiclave, on the

surface and cutting edge of stainless steel and carbon steel curets. Eight carbon steel and six stainless steel Columbia #13/14 curets were cleaned or sterilized in a series of treatments by one of the following methods: ultrasonic cleaning, chemiclave sterilization, and autoclave sterilization. In addition, two carbon steel instruments were placed in an anticorrosive dip followed by autoclave. The blades of the curets were examined and photographed with the scanning electron microscope (SEM) at 200 X and 1000 X for baseline and after 2, 4, 8, 16, and 32 treatment cycles. Photographs were evaluated for visible change in surface appearance by four examiners independently. Photographs for each treatment time were compared to the baseline (pretreatment) photographs for the same instrument. Changes observed were: surface pitting, corrosion products as additions to the surface, edge deterioration, or loss of structure. The study showed that the stainless steel curets showed slight or no change after the three treatments. Carbon steel curets were affected by all treatments. The chemiclave produced slight change. The autoclave produced slight change after the fourth treatment cycle and moderate to major change after the eighth cycle. Those carbon steel instruments treated by anticorrosive dip followed by the autoclave showed pronounced change after 16 or 32 cycles. The most varied results were from the ultrasonic cleaner; one blade showed moderate to major change after only four cycles, the other showed slight change at that point and major change only after 32 cycles. It was concluded that with stainless steel curets, ultrasonic cleanings or sterilization with autoclave or

chemiclave could be used without visibly affecting the cutting edge. With carbon steel instruments, chemiclave is the least damaging sterilization method followed by anticorrosive treatment before autoclaving. Use of the autoclave without the anticorrosive pretreatment or use of an ultrasonic cleaner negatively affects the integrity of the surface and cutting edge of these instruments.

Hurtt CA, Rossman Le[16] in 1996 did a study comparing different methods of sterilizing hand files. The methods used were salt sterilization, glutaraldehyde and autoclave. Six test groups of each 15 files were studied using Bacillus stearothermophillus as the test organism. Groups were "sterilized" by glutaraldehyde immersion for 12 hours, steam autoclaving, and various techniques and timings of salt sterilization, with few files submerged completely and few only to the depth of the handle. The study concluded that only proper steam autoclaving produced completely sterile instruments and that salt sterilization and glutaraldehyde solutions may not be adequate sterilization methods for endodontic hand files and should not be relied on to provide completely sterile instruments.

Haikel .Y et al[11] in 1996 did a study to evaluate the various effects of cleaning, chemical disinfection and sterilization on the cutting efficiency of three file designs (Unifile, Flexofile and H-file). A total of 390 files were divided into 39 groups, each consisting of 10 files. There were 11 groups for each file design plus three control

groups (one from each file design). The different methods used were: ultrasonic cleaning for 4 and 6 cycles of 15 min, disinfection - Sodium hypochlorite for 12hrs and 48hrs and Ammonia for 1hr and 4hrs, sterilization – Chemiclave for 5 and 10 cycles of 20 min at 134°C, Poupinel for 5 and 10 cycles of 120min at 180°C and glass bead for 10 and 40 cycles of 40 seconds at 250°C. Each endodontic instrument underwent 50 cuts and each cut was made on a new surface of the two Plexiglas plates. The cutting efficiency was evaluated as the mass of Plexiglas cut per unit of energy expanded by the instrument in microgram/Joule. The mass of Plexiglas cut was measured simply by direct weigh methods before and after 50 cuts. The results showed that after Poupinel sterilization, the lowest reduction in cutting efficiency was seen in Unifiles and the greatest reduction in cutting efficiency in H-files. After chemiclave sterilization, greatest reduction in Unifiles and lowest reduction in Flexofiles. After bead sterilization, greatest reduction in H-files and lowest reduction in Unifiles. Similarly after ultrasonic cleaning and chemiclave, reduction in cutting efficiency is more or less same in all the three file designs. This study concluded that the greatest reduction in cutting efficiency was in the files sterilized after chemiclave sterilization.

Haikel .Y et al[12] in 1997 did a study to test and compare the values of torsional moment, torsional angular deflection, bending moment and permanent angular deflection of three designs of root canal files (Unifile, Flexofile and H-file) before and after cross-infection

treatment procedures. A total of 390 files were divided into 39 groups, each consisting of 10 files. There were 11 groups for each file design plus three control groups (one from each file design). The sterilization techniques used in the study were: ultrasonic cleaning for 4 and 16 cycles of 15min, chemical disinfection with NaOCl (2.5%) for 12 and 48 hrs and with Ammonia (5%) for 1 and 4 hrs, and sterilization methods – chemiclave for 5 and 10 cycles of 20 min at 134°C, Poupinel (dry heat) for 5 and 10 cycles of 2 hrs at 180°C and glass beads for 10 and 40 cycles of 40 sec at 250°C. The results showed that Unifile was most resistant to fracture (i.e. highest torque resistance), compared with other two files. On the basis of permanent angular deformation, it was found that Unifile also had the greatest initial strength, followed by H-File, then Flexofile. Based on the relative bending moment values, Flexofile had the highest stiffness value (i.e. lowest flexibility).

Silvaggio et al[30] in 1997 did a study to determine whether heat sterilization adversely effects the torsional properties of rotary nickel-titanium files, making them more prone to fracture under torsional stress. Nine hundred files of sizes 2 through 10 Profile Series with 29.04 taper were divided into groups of 10 files each and sterilized 0, 1, 5, or 10 times in the steam autoclave or dry heat sterilizer. Then, they were subjected to torsional testing in a Torquemeter Memocouple. Complete data were collected for sizes 2 through 7, but not for sizes 8 through 10 because their torque resistance exceeded

the testing limits of the Torquemeter Memocouple. A one-way analysis of variance was used to compare all experimental groups in sizes 2 through 7 with their unsterilized controls ($p < 0.05$). Fifty-four comparisons were made for torsional strength and 54 for rotational flexibility. Significant changes occurred in 10 files for torsional strength and in 10 files for rotational flexibility. Eight of 10 changes in torsional strength were increases. Fifty-two of 54 (96.3%) comparisons for torsional strength and 47 of 54 (87%) for rotational flexibility showed a significant increase or no change. Therefore, heat sterilization of rotary nickel-titanium files up to 10 times does not increase the likelihood of instrument fracture.

Haddad et al[10] in 1997 did a study to determine if the positioning of instruments at the centre or edge of a salt sterilizer results in differential sterilization effectiveness, and to compare the effectiveness of salt sterilizers relative to glass bead sterilizers. 60 endodontic reamers were used for this study and they were contaminated with Bacillus stearothermophilus spore suspension. They were then sterilized for different periods of time and at different positions in the sterilizers. Each experiment included positive and negative controls. The results showed that better sterilization is achieved at the edge of the chamber than at the centre, and that salt sterilizers are more effective than glass bead sterilizers for a given period of time (15 seconds).

Velez et al[34] in 1998 studied the ability to sterilize endodontic files inserted into synthetic sponges. Two hundred and forty size 40 K-type endodontic files; 25mm in length were cleaned in an ultrasonic bath. The sponges were then divided into 4 groups of 15 sponges: positive control, negative control, dry heat and steam autoclave. These groups were subdivided into 5 subgroups of 3 and submitted to 1 to 5 sterilization cycles. Three groups of 60 files each were inoculated with Bacillus stearothermophillus. Sixty noncontaminated instruments were used as negative control. Four files were inserted in each sponge and the sponges were subjected to 5 cycles by either autoclave at 121°C and 15 pounds psi for 20 min, or dry heat at 160°C for 60 min. After each cycle, each file and a portion of sponge surrounding the file were transferred aseptically to tubes containing tryticase soy broth culture medium for bacteriological analysis and incubated at 55°C for 7 days. The tubes were examined and evaluated for B. stearothermophillus growth. If microorganisms grew, they were plated and identified. The results showed that all sponges and contaminated files used as positive controls were positive for bacterial growth and all sponges and non-contaminated files used as negative controls were negative. There was 100% sterility of files of those inserted in sponges and placed in the steam autoclave for sterilization and only 96.7% sterility with contaminated instruments inserted in the sponges and placed in the dry heat (Driclave).

Canalda-Sahli et al[5] in 1998 did a study to assess the effect of dry-heat or autoclave sterilization on the resistance to fracture in torque and angular deflection and the resistance to bending of K-type files made of nickel-titanium, titanium or stainless steel. Ten K-files of each, from size 25 to 40, were tested. Sterilization with dry-heat and autoclave slightly decreased the flexibility of files made of stainless steel and nickel-titanium for most of the sizes. The files made of titanium showed an increased flexibility after sterilization with autoclave (sizes 30 and 35) and dry heat (sizes 30,35 and 40). Resistance to fracture varied among the five groups of files tested as follows: it decreased in some sizes of stainless steel instruments, decreased in all sizes of titanium files assessed by the torsional moment and either increased or decreased in some sizes of nickel-titanium files. Resistance to angular deflection by twisting decreased slightly in stainless-steel files, decreased significantly in titanium files evaluated by the torsional moment and increased or decreased in nickel titanium files. The study concluded that all files tested for torsion and angular deflection after sterilization with an autoclave or dry heat satisfied relevant standards.

Mize et al[19] in 1998 did a study to determine the effect of heat treatment resulting from autoclave sterilization procedures on the cyclic fatigue properties of rotary Ni-Ti endodontic instruments. 280 size 40 light speed instruments were used without a cutting head to facilitate insertion and fixation within the collect of a dynamometer.

Two experiment protocols were conducted using the presterilized instruments to examine the effect of a single sterilization (part I) or repeated sterilization (part II) on the cycles to failure. The instruments were cycled in artificial canals that were constructed from stainless steel tubes. The two artificial canals were constructed with a 30-degree angle of curvature, and either 2 or 5 min radius of curvature. In part 1, instruments were cycled to 25%, 50%, or 75% of the predetermined cycles-to-failure limit. There were 56 instruments in each of the three groups. Each group was then divided; 28 instruments were sterilized and the other 28 were not. All instruments were then cycled to failure. All groups were cycled, at both the 2 and 5 mm radii and this produced 12 groups with 14 instruments in each group. For part II, 56 instruments were cycled to 25% of the predetermined cycles-to-failure limit, with 28 using the 2 mm radius canal and 28 using the 5 mm radius canal. The groups were divided; 14 instruments were sterilized and the other 14 were not. The instruments were then cycled to 25% of failure again and, sterilized or not sterilized; this procedure was repeated until the instruments failed and this produced 4 groups with 14 instruments in each group. The study resulted that autoclave sterilization, either single or repeated, did not increase total cycles to failure when comparing instruments cycled to failure at a similar radius. Significant differences on cycles to failure was observed when instruments cycled to failure in the artificial canal with 2mm radius were compared with instruments cycled to failure in the artificial canal with 5mm radius. The study

concluded that heat treatment as a result of autoclave sterilization does not extend the useful life of nickel-titanium instruments.

Rapisarda et al[27] in 1999 did an in vitro study to evaluate the behavior of nickel-titanium rotary instruments under repeated sterilization cycles. 36 ProFile instruments, 18 with a taper of .04 and 18 with a taper of .06, were divided into 3 groups of 12 files each. The groups were exposed to 14 cycles of sterilization for 30 minutes; 7 cycles of sterilization for 30 minutes. The third group was not sterilized and served as a control group. Chemical compositions of the near surface layers of samples of each group were determined by means of Auger spectroscopy. The results showed that the instruments that underwent the greatest number of sterilizations showed in-depth distributions of chemical composition that were different from those seen in the control group; this was the result of greater amounts of titanium oxide on the surfaces of the sterilized instruments. The group of files that underwent 14 cycles of sterilization showed a decrease in cutting efficiency in comparison with those of the control group. It was concluded that repeated sterilizations under autoclave alter the superficial structure of nickel-titanium files.

Yared et al[36] in 1999 did a study to evaluate cyclic fatigue of Profile Ni-Ti rotary instruments (PRI's) after dry heat sterilization and

up to 10 times simulated clinical use. : Instruments of size 15-40 were used in a crown-down technique. Three groups were included in this study. In groups 1 and 2, each set of instruments was used in five and 10 canals, respectively. Group 3 was the control group and sodium hypochlorite at a concentration of 2.5% was used as an irrigant. The study showed that size 40 files demonstrated the lowest incidence of rotations to breakage and there was significant difference among different file sizes within each group. The study concluded that that dry heat sterilization and simulated clinical use in the presence of sodium hypochlorite did not lead to a decrease in the number of rotations to breakage of the files.

Hilt et al[13] in 2000 tested the hypothesis that multiple sterilizations of endodontic stainless-steel and nickel-titanium files will lead to a continuous decrease in the resistance of files to separation by torsion. One hundred stainless-steel and 100 nickel-titanium no.30 K-type files were divided into 20 groups of 10 and sterilized in increments of 10 cycles, using a full cycle and a fast cycle autoclave. These files were tested by twisting each of them in a clockwise direction until fracture (torque g-cm). Samples of the fractured files were embedded in an epoxy resin and polished for Knoop hardness tests. In addition, the samples were chemically etched to reveal changes in microstructure. The findings of this study indicated that neither the number of sterilization cycles nor the type of

autoclave sterilization used affects the torsional properties, hardness, and microstructure of stainless steel and nickel-titanium files.

Ueno et al[34] in 2000 did a study to see the effectiveness of a new sterilization technique – oxygen plasma for endodontic files. The endodontic files used in the study were contaminated with Escherichia coli, Pseudomonas aeruginosa or Staphylococcus aureus. The files were subjected to sterilization by oxygen plasma formed through a continuous electric discharge of 75 mA. The results demonstrated that files contaminated with Pseudomonas aeruginosa and Escherichia coli were sterilized within a 1-minute exposure, while Staphylococcus aureus required 10 minutes. The study concluded that Gram-positive microorganisms such as Staphylococcus aureus require more time of plasma bombardment than Gram-negative microorganisms.

RajKumar et al[26] in 2001 did a study to investigate the efficacy of two accepted methods of sterilizing endodontic instruments: (1) Autoclaving of endodontic files when placed in (a) Endodontic instruments box (b) Synthetic sponge (2) The efficacy of glass bead sterilizer at different time intervals. The test sample of 80 files divided into Nine groups, seven groups containing 10 files in each group contaminated with Bacillus stearothermophillus, one group containing

five contaminated files which were used as a positive control and last group containing 5 uncoated files which were used as a negative control. The files in each group were sterilized with different method and time interval and checked for any growth after incubating them for 24 hours at 45°C. The results indicated that autoclaving in both endodontic instrument box, synthetic sponge and sterilized in glass bead sterilizer for 45 seconds after wiping with spirit soaked gauze for 10 seconds, there was total sterility. When the files were wiped for 10 seconds with gauze soaked with spirit and sterilized in glass bead sterilizer for 10,15, 20 and 40 seconds, there was incomplete sterilization to the range of 30%, 44%, 66% and 92%. The study concluded that files should be autoclaved in either an endodontic instrument box or a synthetic sponge at 121°C for 15 pounds pressure to achieve complete sterilization. It also concluded that glass bead sterilization of files for 45 seconds at 240°C after wiping them with 2 X 2 inch fold gauze soaked with spirit could be considered as a chair side alternative.

Schafer[29] in 2002 did a study to investigate the alterations in cutting efficiency when conventional and titanium nitride (TiN) coated nickel-titanium (NiTi) K-files were exposed to repeated sterilization using an autoclave. A total of 96 NiTi K-files (size 35) were randomly divided into two groups (A and B) of 48 instruments each. The instruments of group B were exposed to physical vapor deposition (PVD) creating a coating of a TiN layer; the files of group A were not

coated. The instruments of groups A and B were randomly divided into four subgroups of 12 instruments each. A.1/B.1: Instruments were exposed to five cycles of sterilization. A.2/B.2: Instruments were exposed to 10 cycles of sterilization. A.3/B.3: Instruments were immersed in NaOCl for 30 min, rinsed in water, and exposed to five cycles of sterilization. A.C/B.C: Instruments were not sterilized (controls). The cutting efficiency of all files was determined by means of a computer-driven testing device. Special plastic samples with cylindrical canals were used and the maximum penetration depth of the files into the lumen was assessed. The results showed that the TiN-coated instruments of groups B.1, B.2 and B.3 did not show any significant difference in comparison with the penetration depths of the controls whereas the uncoated files of groups A.1, A.2 and A.3 displayed significantly lower maximum penetration depths when compared to the control files. The study concluded that repeated sterilization under autoclave or exposure to sodium hypochlorite (NaOCl) prior to sterilization did not alter the cutting efficiency of PVD-coated NiTi K-files.

Testarelli et al[32] in 2003 did a study to evaluate the mechanical resistance of nickel-titanium files before and after sterilization procedures. Thirty 02, 04, 06 tapered Hero size 30 new nickel-titanium instruments were chosen and divided into 3 groups. Group A was not sterilized and used as control group. Group B files were first sterilized with chemiclave for 10 cycles of 20 minutes at 124

inverted exclamation mark C. Group C files were sterilized with glass beads for 10 cycles of 20 seconds at 250 inverted exclamation mark C. They were then tested for torsional resistance, angle of torque and angle at breakage (45 inverted exclamation mark). The results showed that differences among the 3 groups were statistically not significant for both tests. The study concluded that repeated sterilization procedures do not adversely affect the mechanical resistance of Hero nickel-titanium files.

Parashos et al[23] in 2004 did a study to develop a clinically practical cleaning protocol for rotary nickel-titanium (Ni-Ti) endodontic files prior to sterilization. The experiments involved three components of mechanical and chemical removal of root canal debris from the files: the use of sponges soaked with chlorhexidine to remove gross debris, pre-soaking, and ultrasonification. After cleaning, the files were immersed in Van Gieson's solution and examined under magnification for stained debris. The results were that there were no instances of visible debris and all files appeared clean after all cleaning sequences. The study concluded that combining elements of the most effective cleaning sequences resulted in a cleaning protocol that predictably produced clean files.

Whittaker et al[34] in 2004 did a study to see the effective use of a commercial gas plasma etcher in the cleaning of endodontic files. The cleaned, sterilized, files were screened, using scanning electron

microscopy and energy-dispersive X-ray analysis, to determine the level of contamination before plasma cleaning. The files were then exposed for a short-term to low-pressure oxygen-argon plasma, before being re-examined. In all cases, the amount of organic material was reduced to a level below the detection limit of the instrument. This study suggested that plasma cleaning offered a safe and effective method for decontamination of dental instruments, thus reducing the risk of iatrogenic transmission of disease during dental procedures.

MATERIALS AND METHODS

MATERIALS AND METHODS

This study was done to investigate the efficacy of four accepted methods of sterilizing endodontic files.

Part of the study was done at Dept. of Microbiology, Malar Hospital, Chennai and the CO_2 laser system part of the study was done at KKR ENT Hospital, Chennai.

The study was carried on 100 K-files, 21mm long and of size 25. Of these 20 files were taken as control group and the remaining 80 files were divided into 4 groups of 20 files in each group and they were tested for the efficacy of sterilization with different methods: autoclave, glass bead, glutaraldehyde and laser.

MATERIALS / EQUIPMENTS

The materials / equipments used in this study were

1. Autoclave
2. Surgical laser unit
3. Glass bead sterilizer
4. Glutaraldehyde
5. Incubator
6. Endodontic instrument box
7. Wire cutters

8. Tweezers
9. Bunsen burner
10. Petri dishes
11. Autoclavable pouches
12. Test-tubes
13. Methylated spirit
14. Gauze (2 x 2 inch)
15. Thioglycollate medium
16. Bacillus stearothermophillus spores
17. Glass slides
18. Light microscope
19. Sugar containing test tubes

METHODOLOGY

100 K-files of size 25, 21mm long were taken for this study.

All the 100 files included in the study were pre-sterilized in a endodontic instrument box by autoclaving for 30 minutes at 121° C at 15 pounds pressure, for standardization to eliminate any bias.

The test files were divided into 5 groups of 20 files in each group and labeled as A (autoclave), B (glass bead), G (glutaraldehyde), L (CO_2 laser) and C (control) and were numbered 1 to 20.

The spore suspension was prepared by immersing the commercially available bacillus stearothermophilus strips into thioglycollate medium broth and incubated at 55°C for 48 hours. Growth that occurred in the test tube was confirmed by doing Gram's stain that showed the presence of gram-positive bacillus stearothermophilus.

All the pre-sterilized files were contaminated with Bacillus stearothermophillus (Fig.2) in a sterile petri dish for 5 minutes (Fig.3, Fig.4). After 5 minutes of immersion, the files were transferred to another sterile petri dish under a vacuum hood safety with the help of a sterile tweezer, following which the files were dried in an incubator for 10 minutes at 37°C and stored in an endodontic instrument box till they were sterilized by different methods.

Group A containing 20 contaminated files were placed in an endodontic instrument box and subjected to autoclave at 121°C for 15 minutes under 15 pounds pressure. (Fig.5)

Group B containing 20 contaminated files were taken in 4 batches of 5 files each and wiped for 10 seconds with a 2 X 2 gauze soaked with surgical spirit and were placed in the periphery of the glass bead sterilizer (Fig.6) and sterilized for 45 seconds at 240°C.

Group G containing 20 contaminated files were placed in a sterile plastic container containing 2.4% glutaraldehyde solution (Fig.7) and is left in it for 12 hours.

Group L containing 20 contaminated files were irradiated for 3 seconds per surface at 10 watts using CO_2 laser system (Fig.8). The laser beam was moved along the length of the instrument during the 3-second period. A sterile tweezer was used to hold the handle of the file and change the surface for exposure.

After completion of sterilization of the files as described, the shaft of the instrument was removed from the handle by means of a sterile autoclaved wire cutter and each file was placed in separate tubes containing thioglycollate medium with the help of a sterile tweezer.

Group C containing control group of 20 contaminated files was put in separate tubes containing thioglycollate medium by the method described above without doing any sterilization.

The test tubes containing files were labeled with the date and were kept for incubation at 55°C for 3 days (Fig.9). After 3 days the test tubes were removed from the incubator and each test tube was checked for any turbidity in the test tube. Presence of turbidity in a test tube indicated the presence of Bacillus stearothermophilus and that the particular file was not sterilized completely. The test tubes

were then further kept for incubation at 55°C till 21 days and again checked for growth in the similar method as described above.

The test tubes with turbidity were checked and confirmed for the presence of microorganism by viewing under light microscope after doing Gram's stain and culture test. The specificity of Bacillus stearothermophilus stains was confirmed with sugar test.

The observations were tabulated and subjected to statistical evaluation using Trend-Chi's square test.

Fig.1 Armentarium

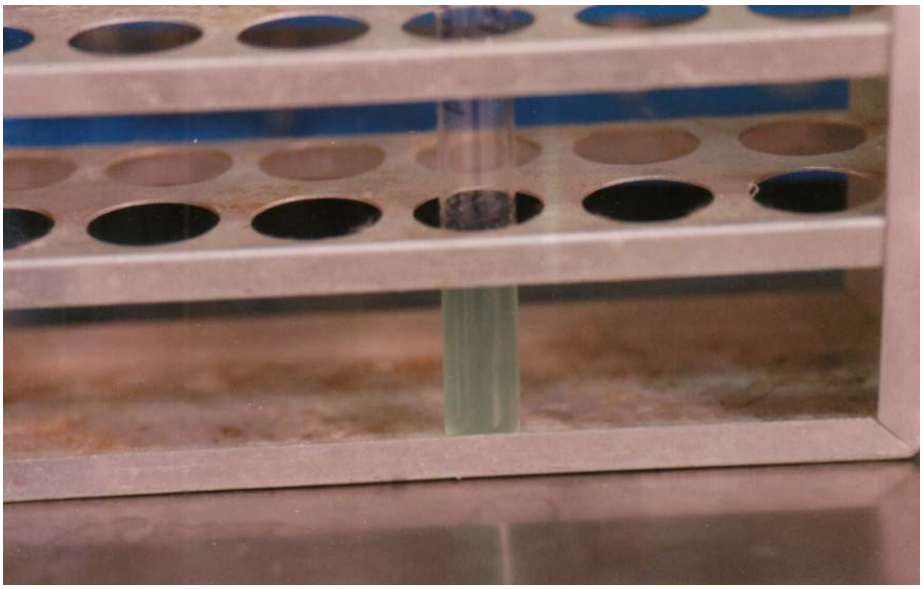


Fig.2 Bacillus stearothermophillus

Fig.3 Contamination under biosafety hood

Fig.4 Contamination of files in Petri-dish

Fig.5 Autoclave

Fig.6 Glass Bead

Fig.7 Glutaraldehyde

Fig. 8 CO_2 Laser Unit

Fig. 9 Incubator

RESULTS

RESULTS

This study was done to determine the efficacy of sterilizing endodontic files by different methods – autoclave, glass bead, glutaraldehyde and laser. The endodontic files were sterilized after contamination with bacillus spores.

The study showed that the endodontic files sterilized by autoclaving in an instrument box at 121°C for 15 minutes under 15 pounds pressure (Group A) showed total sterility. This method of sterilizing the endodontic files achieved complete sterilization. (Fig.'s 10,11,12)

The files subjected to sterilization by glass bead sterilizer after wiping for 10 seconds with a 2 X 2 gauze soaked with surgical spirit and sterilized for 45 seconds at 240°C (Group B) showed presence of turbidity in 2 test tubes. Incomplete sterilization to the range of 10% was observed when the files were sterilized in glass bead sterilizer. (Fig.'s 13,14,15)

The endodontic files sterilized by immersing in glutaraldehyde for 24 hours (Group G) showed sterilization upto only 80%. This method showed contamination of 4 files after incubation. (Fig.'s 16,17,18)

The files on sterilization by CO_2 laser for 3 seconds per surface at 10 watts (Group L) showed 100% sterility. There was total sterility seen by this method of sterilization. (Fig.'s 19,20,21)

The control group (Group C) where the files after contamination were not sterilized by any method showed growth in all the test tubes. (Fig.'s 22,23,24)

Statistical analysis of the 4 sterilized groups showed a statistical significance ($p \leq 0.05$). This is shown in Table 1.

The statistical analysis of the sterilized groups with that of the control group showed that it is significant ($p \leq 0.05$).

On comparing the different groups among themselves, it was seen that there was no statistical significance among them.

The results are summarized in tabular columns below.

Table 2 shows the comparison of groups sterilized by autoclave (Group A) and glass bead (Group B). ($p = 0.487$)

Table 3 shows the comparison of groups sterilized by autoclave (Group A) and glutaraldehyde (Group G). ($p = 0.106$)

Table 4 shows the comparison of groups sterilized by autoclave (Group A) and laser (Group L). ($p = 0$)

Table 5 shows the comparison of groups sterilized by glass bead (Group B) and glutaraldehyde (Group G). ($p = 0.376$)

Table 6 shows the comparison of groups sterilized by glass bead (Group B) and laser (Group L). ($p = 0.147$)

Table 7 shows the comparison of groups sterilized by glutaraldehyde (Group G) and laser (Group L). (p = 0.106)

TABLE 1

GROUPS	GROWTH		NO GROWTH		p VALUE
	NUMBER n	PERCENTAGE (%)	NUMBER n	PERCENTAGE (%)	
A	—	—	20	100%	0.048
B	2	10%	18	90%	
G	4	20%	16	80%	
L	—	—	20	100%	
C	20	100%	—	—	

TABLE 2

GROUPS	GROWTH		NO GROWTH	
	NUMBER n	PERCENTAGE (%)	NUMBER n	PERCENTAGE (%)
A	—	—	20	100%
B	2	10%	18	90%

p = 0.487

TABLE 3

GROUPS	GROWTH		NO GROWTH	
	NUMBER n	PERCENTAGE (%)	NUMBER n	PERCENTAGE (%)
A	—	—	20	100%
G	4	20%	16	80%

p = 0.106

TABLE 4

GROUPS	GROWTH		NO GROWTH	
	NUMBER n	PERCENTAGE (%)	NUMBER n	PERCENTAGE (%)
A	—	—	20	100%
L	—	—	20	100%

p = 0

TABLE 5

GROUPS	GROWTH		NO GROWTH	
	NUMBER n	PERCENTAGE (%)	NUMBER n	PERCENTAGE (%)
B	2	10%	18	90%
G	4	20%	16	80%

p = 0.376

TABLE 6

GROUPS	GROWTH		NO GROWTH	
	NUMBER n	PERCENTAGE (%)	NUMBER n	PERCENTAGE (%)
B	2	10%	18	90%
L	2	10%	20	100%

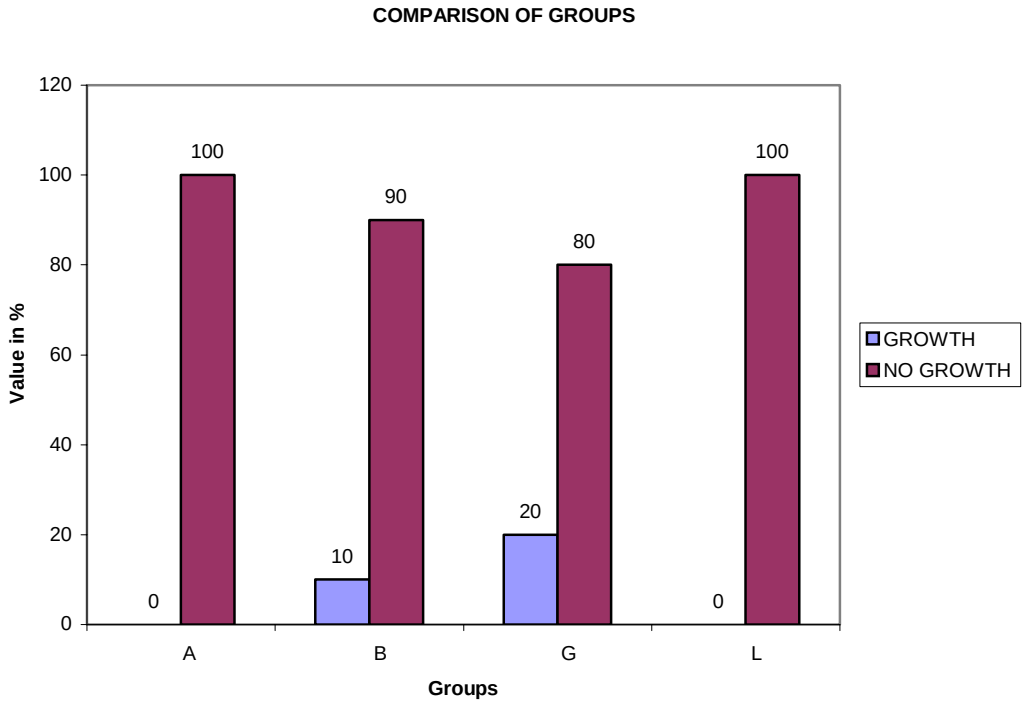
p = 0.147

TABLE 7

GROUPS	GROWTH		NO GROWTH	
	NUMBER n	PERCENTAGE (%)	NUMBER n	PERCENTAGE (%)
G	4	20%	16	90%
L	2	10%	20	100%

p = 0.106

Microorganisms were identified as Bacillus stearothermophillus rods using dark-field microscopy after gram's staining (Fig.25). The sugar test showed no fermentation, which confirms the presence of gram-positive bacilli. (Fig.26)


COMPARISON OF GROUPS
120
100
80
60
40
20
0
Value in %
100
90
80
100
0
10
20
0
A
B
G
L
Groups
GROWTH
NO GROWTH

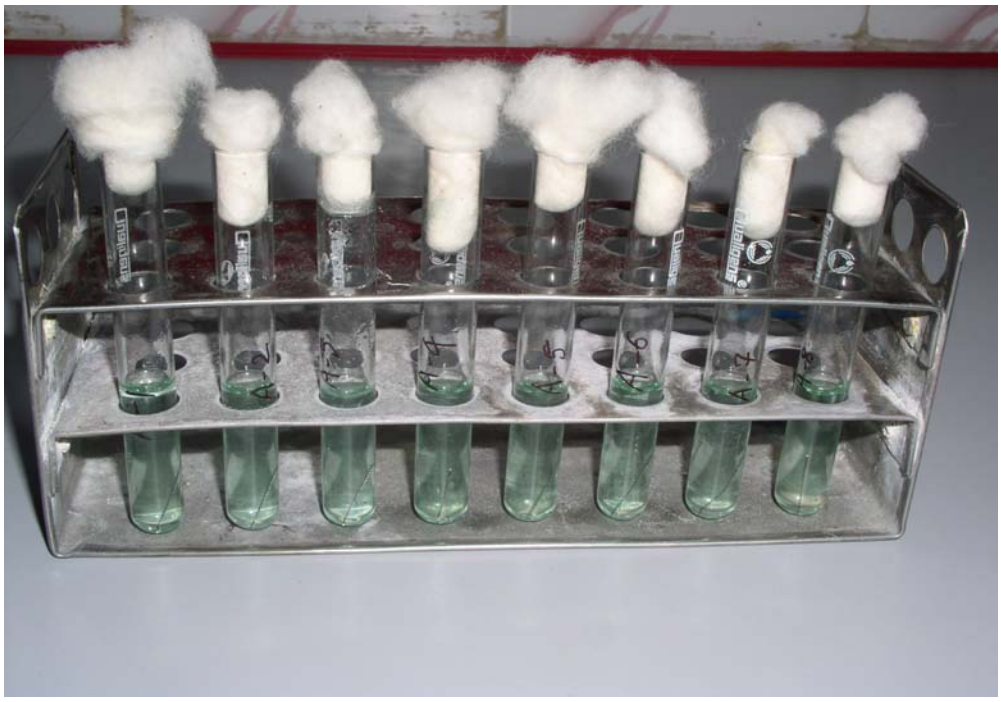


Fig. 10 Test-tubes after autoclave sterilization (1-8)

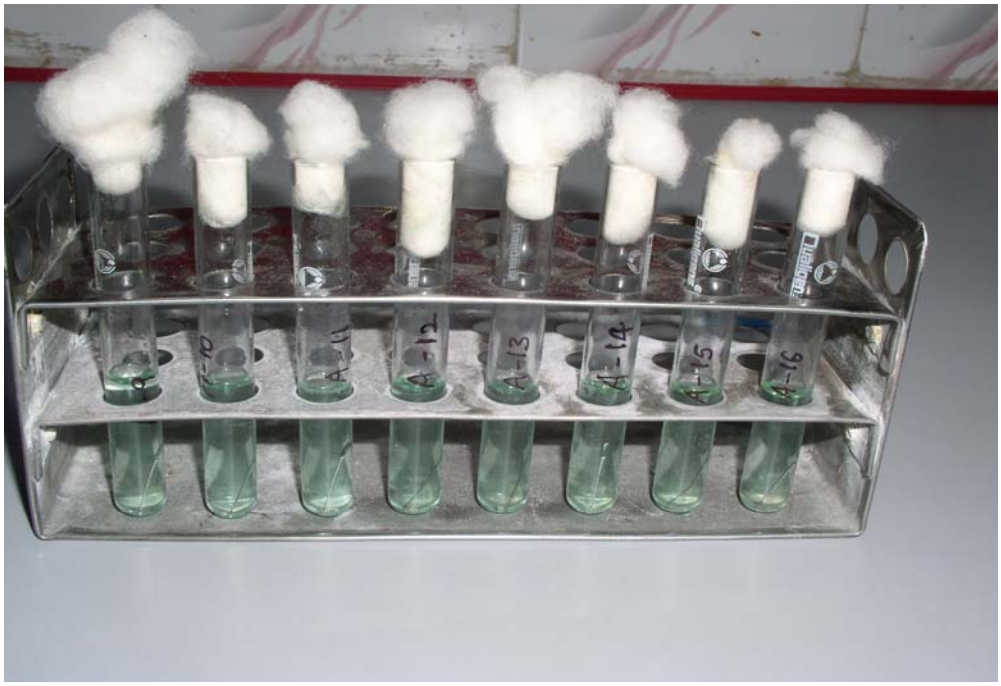


Fig. 11 Test-tubes after autoclave sterilization (9-16)

Fig. 12 Test-tubes after autoclave sterilization (17-20)

Fig. 13 Test-tubes after glass bead sterilization (1-8)

Fig.14 Test-tubes after glass bead sterilization (9-16)

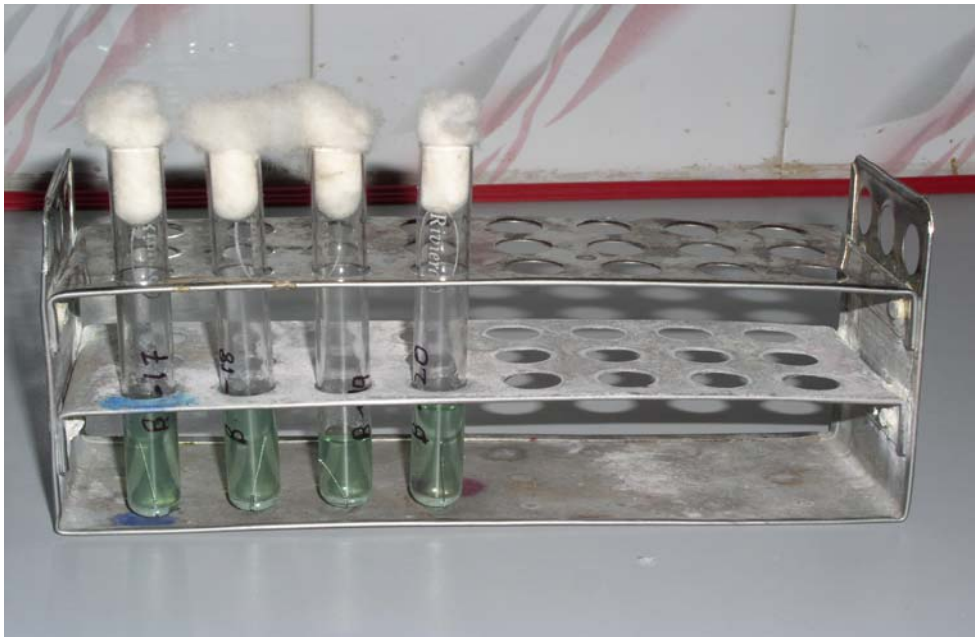


Fig.15 Test-tubes after glass bead sterilization (17-20)

Fig.16 Test-tubes after glutaraldehyde sterilization (1-8)

Fig. 17 Test-tubes after glutaraldehyde sterilization (9-16)

Fig.18 Test-tubes after glutaraldehyde sterilization (17-20)

Fig.19 Test-tubes after laser sterilization (1-8)

Fig.20 Test-tubes after laser sterilization (9-16)

Fig.21 Test-tubes after laser sterilization (17-20)

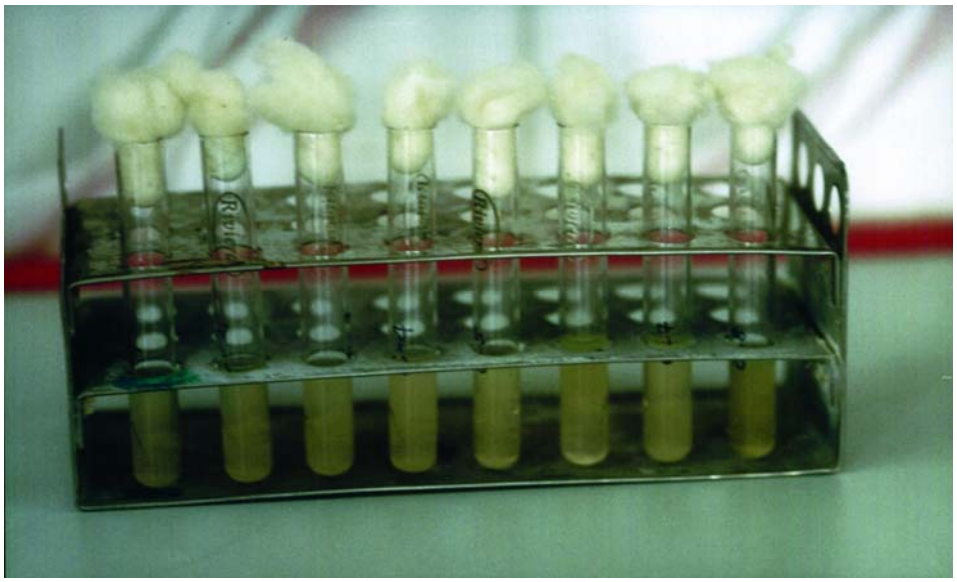


Fig.22 Test-tubes of Control group (1-8)

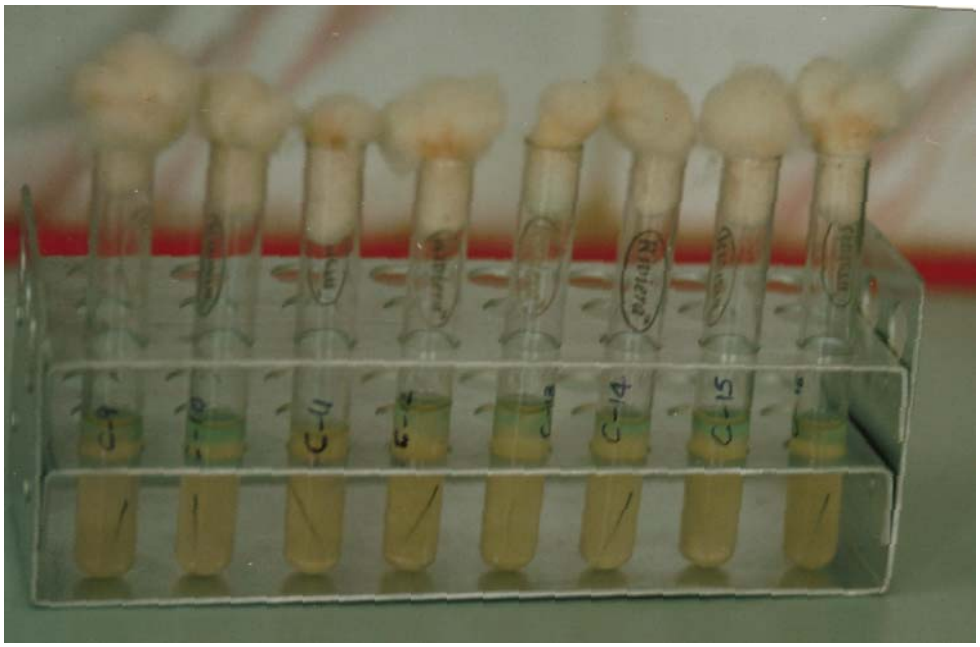


Fig.23 Test-tubes of Control group (9-16)

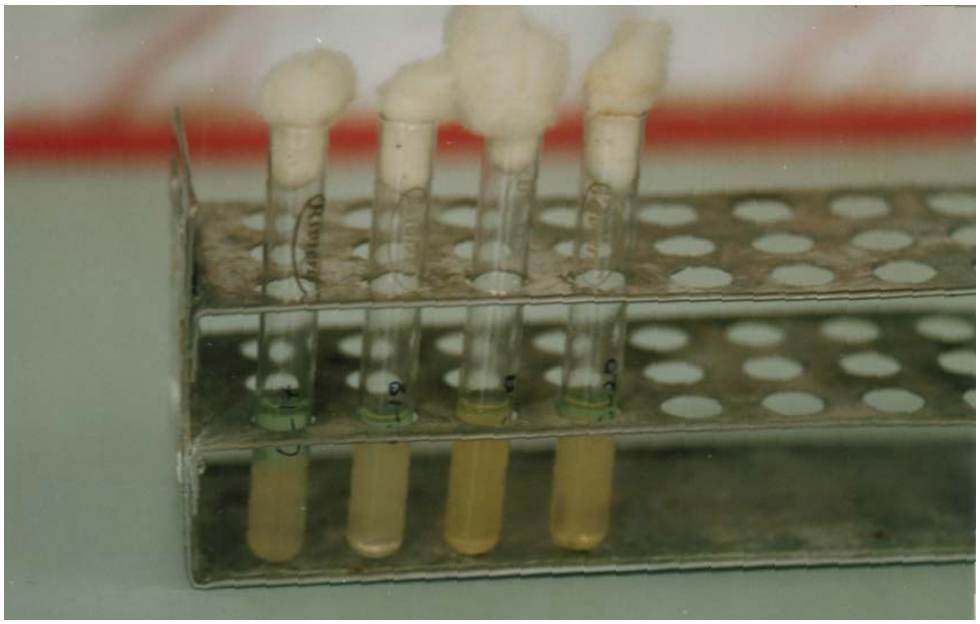


Fig.24 Test-tubes of Control group (17-20)

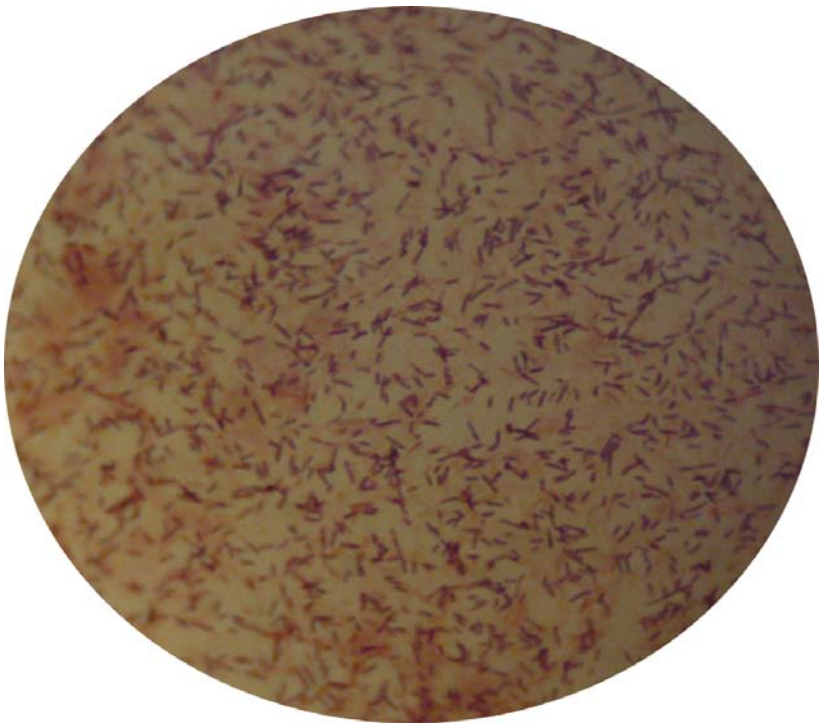


Fig.25 Microscopic view of Bacillus sterothermophillus

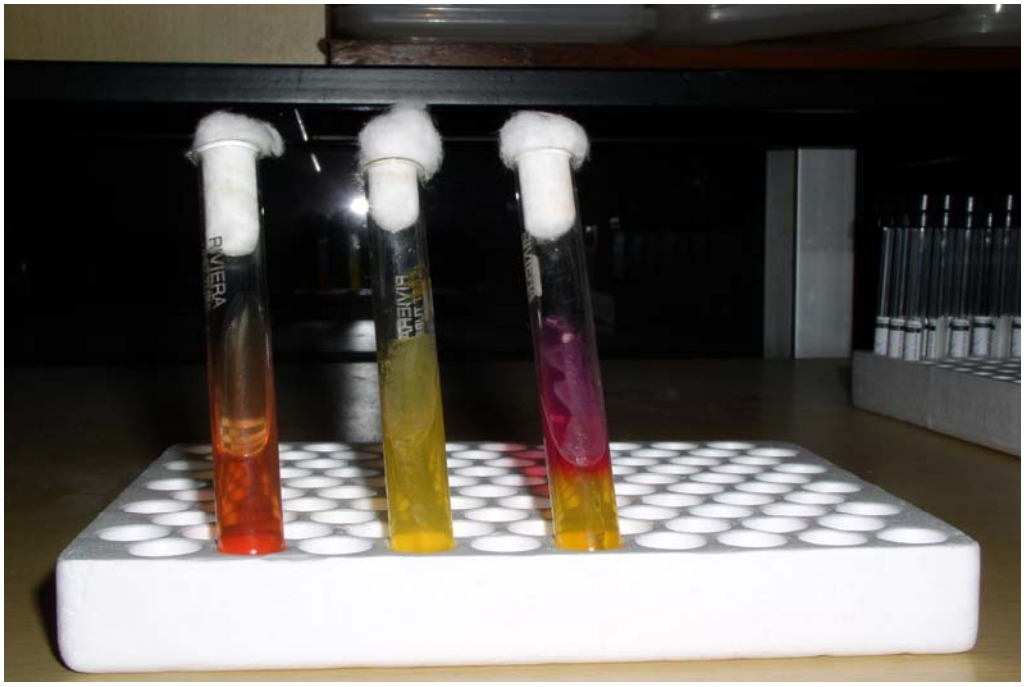


Fig.26 Test-tubes of sugar test

DISCUSSION

DISCUSSION

This study was done to evaluate a fast and effective method to sterilize endodontic files in a clinical set up.

100 K-files of size 25, 21mm long were taken for this study and were presterilized before contamination with bacillus stearothermophillus. 4 different methods- autoclave, glass bead, glutaraldehyde and laser were used in this study to sterilize by taking 20 files for each method. The remaining 20 files were not sterilized after contamination and were taken as control group.

Instrument sterilization involves four distinct processes: presterilization, cleaning, sterilization and aseptic storage.

There are three principle methods of sterilization of instruments available: steam under pressure (autoclave), dry heat and chemiclave. Another method of sterilization – laser is also available but not used popularly. In this study, all these are tested to see which is effective method that can be easily followed in a dental office. Instruments should be first cleaned of debris regardless of the method used to sterilize them. They should be wiped clean by squeezing the instrument blade with a 2 X 2 gauze or cotton roll, moistened with hydrogen peroxide or alcohol, while withdrawing the instrument, using a counter-clockwise rotary motion before subjecting them to sterilization[9]. Surgical spirit or methylated spirit, which contains methyl alcohol, can be used as a fungicidal or

sporicidal agent[2]. Hubbard Jr. et al[15] showed that all gauze wipings showed reductions of microorganisms greater then 90 percent.

Files have complicated design. Debris accumulates between the flutes in an area, which is relatively inaccessible for cleaning. Inspite of cleaning the instruments with sterile gauze the debris still remains and interferes with the sterilization of the instruments[26].

The spores of bacillus stearothermophillus used to contaminate the files in this study are heat-resistant bacterial spore used in many of the previous researchers[26, 16, 34, 10].

Many methods have been advocated for sterilization of endodontic instruments. Steam autoclaving and glass bead sterilizers are among the commonly recommended method of sterilization. Boyd[4] stated the moist heat generally kills the microorganism by coagulations of proteins. However, coagulation occurs only when over kill conditions are attained. Less drastic changes such as inactivation of enzymes, changes in nucleic acids and cytoplasmic membrane alterations probably kill the microorganisms before coagulation occurs[4]. The present study indicated that complete sterilization was possible by autoclaving the instruments in an endodontic box. This is significant with studies done by other researchers like Rajkumar et al[26,] Hurtt et al[16,] Velez et al[34]. Boyd et al[3] experimented endodontic files contaminated with spores of Bacillus stearothermophillus which were inserted in synthetic sponge and concluded that synthetic sponge does not impede autoclaving process.

Glass bead sterilizer works on the principle of intense dry heat. Heat causes damage to both vegetable and spore forms of bacteria. Thermo sensitivity of biomolecules to dry heat is much less than moist heat. Damaging alterations of proteins by dry heat are the result of oxidation, desiccation and changes in osmotic pressure owing to evaporation of moisture[d3]. Dry heat is slower and requires temperatures higher than those used in moist heat sterilization. This study showed that sterilization by glass bead sterilizer was upto only 90% and that total sterility was not found even after sterilizing for 45 seconds at 240°C. Incomplete sterilization was in the range of 10%. The present study result was contradictory to previous research done by Rajkumar et al[26]. But it was the same as the research done by Hurtt et al[16] who did the study with salt instead of glass bead.

This present study showed 80% sterilization by immersing the files in glutaraldehyde solution for 24 hours which is similar to the results of study done by Hurtt et al[16] and so glutaraldehyde solution cannot be relied on completely to sterilize endodontic instruments.

The present study indicated that CO_2 laser was used because since CO_2 laser is starting to become a common usage nowadays in the dental office for periodontal surgery, endodontic instrumentation and so is an effective method of sterilizing endodontic files and can be followed in a dental clinic. Nammour et al[22] in a study concluded that CO_2 laser had a

important potential for sterilization. Powell et al[25] in a study told that all three lasers (argon, CO_2, and NdYAG) were capable of sterilizing dental instruments, but argon laser is capable of sterilizing at the lowest energy level. The result of this present study is similar to study done by Hooks et al[14] and Adrian & Gross[1].

A multitude of factors are to be considered in prevention of cross contamination. The endodontic instruments should be sterilized effectively before use on different patients to prevent cross contamination. The present study indicates that autoclaving and exposing to laser gives complete sterilization. Glass bead sterilizer can be used as an alternative when the other two methods are not available, but glutaraldehyde cannot be relied on as a method of sterilization.

Though autoclave is effective method for sterilizing endodontic files, the time taken by it to sterilize is more, but lasers can sterilize the endodontic instruments effectively and also fast. As the use of CO_2 laser is becoming common in dental clinic, it can be used as a routine method and also as a chair side method to sterilize endodontic files in a routine clinical practice.

CONCLUSION

CONCLUSION

The following conclusions were drawn from the present study.

1. CO_2 laser and autoclave were showing total sterility and are more efficient than glass bead and glutaraldehyde.
2. Autoclave can be used as a method for sterilization in clinical practice and in advanced clinics laser can be used a chair side method of sterilization.
3. Though laser is an effective method of sterilization, further studies have to determine the cutting efficiency and other mechanical properties of endodontic files after repeated exposure to CO_2 laser.

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